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PATENT APPLICATION

SYNTHESIS OF OLIGONUCLEOTIDE ARRAYS USING PHOTOCLEAVABLE PROTECTING GROUPS

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SYNTHESIS OF OLIGONUCLEOTIDE ARRAYS USING PHOTOCLEAVABLE PROTECTING GROUPS

BACKGROUND OF THE INVENTION

The present invention relates to the area of chemical synthesis. More particularly, this invention relates to photolabile compounds, reagents for preparing the same and methods for their use as photocleavable linkers and protecting groups, particularly in the synthesis of high density molecular arrays on solid supports.

The use of a photolabile molecule as a linker to couple molecules to solid supports and to facilitate the subsequent cleavage reaction has received considerable attention during the last two decades. Photolysis offers a mild method of cleavage which complements traditional acidic or basic cleavage techniques. *See, e.g.*, Lloyd-Williams et al. (1993) *Tetrahedron* **49:**11065-11133. The rapidly growing field of combinatorial organic synthesis (*see, e.g.*, Gallop et al. (1994) *J. Med. Chem.* **37:**1233-1251; and Gordon et al. (1994) *J. Med. Chem.* **37:**1385-1401) involving libraries of peptides and small molecules has markedly renewed interest in the use of photolabile linkers for the release of both ligands and tagging molecules.

A variety of *ortho*-benzyl compounds as photolabile protecting groups have been used in the course of optimizing the photolithographic synthesis of both peptides (*see* Fodor et al. (1994) *Science* **251:**767-773) and oligonucleotides (*see* Pease et al. *Proc. Natl. Acad. Sci. USA* **91:**5022-5026). *See* PCT patent publication Nos. WO 90/15070, WO 92/10092, and WO 94/10128; see also U.S. patent application Serial No. 07/971,181, filed 2 Nov. 1992, and Serial No. 08/310,510, filed September 22, 1994; Holmes et al. (1994) in *Peptides: Chemistry, Structure and Biology (Proceedings of the 13th American Peptide Symposium)*; Hodges et al. Eds.; ESCOM: Leiden; pp. 110-12, each of these references is incorporated herein by reference for all purposes. Examples of these compounds included the 6-nitroveratryl derived protecting groups, which incorporate two additional alkoxy groups into the benzene ring. Introduction of an α-methyl onto the benzylic carbon facilitated the photolytic cleavage with > 350 nm UV light and resulted in the formation of a nitroso-ketone.

Photocleavable protecting groups and linkers should be stable to a variety of reagents (*e.g.*, piperidine, TFA, and the like); be rapidly cleaved under mild conditions; and

not generate highly reactive byproducts. The present invention provides such protecting groups and methods for their use in synthesizing high density molecular arrays.

SUMMARY OF THE INVENTION

According to a first aspect of the invention, novel compounds are provided which are useful for providing protecting groups in chemical synthesis, preferably in the solid phase synthesis of oligonucleotides and polypeptides. These compounds are generally photolabile and comprise protecting groups which can be removed by photolysis to unmask a reactive group. The compounds have the general formulas as shown in Figure 1 and 9.

Another aspect of this invention provides a method of attaching a molecule with a reactive site to a support comprising the steps of:

(a) providing a support with a reactive site;

(b) binding a molecule to the reactive site, the molecule comprising a masked reactive site attached to a photolabile protecting group of the formula as shown in Figure 1, and

(c) removing the photolabile protecting group to provide a derivatized support comprising the molecule with an unmasked reactive site immobilized thereon.

A related aspect of this invention provides a method of forming, from component molecules, a plurality of compounds on a support, each compound occupying a separate region of the support, said method comprising the steps of:

(a) activating a region of the support;

(b) binding a molecule to the region, said molecule comprising a masked reactive site linked to a photolabile protecting group of the formula as shown in Figure 1;

(c) repeating steps (a) and (b) on other regions of the support whereby each of said other regions has bound thereto another molecule comprising a masked reactive site linked to the photolabile protecting group, wherein said another molecule may be the same or different from that used in step (b);

(d) removing the photolabile protecting group from one of the molecules bound to one of the regions of the support to provide a region bearing a molecule with an unmasked reactive site;

(e) binding an additional molecule to the molecule with an unmasked reactive site;

(f) repeating steps (d) and (e) on regions of the support until a desired plurality of compounds is formed from the component molecules, each compound occupying separate regions of the support.

This method finds particular utility in synthesizing high density arrays of nucleic acids on solid supports in either the 3'->5' or 5'->3' directions.

BRIEF DESCRIPTION OF THE DRAWINGS

SUB A2

Figure 1 shows a general outline of the alternative synthesis chemistries and outlines what the general structures for "Y" could be.

SUB A3

Figure 2 shows specific compounds that are preferred within the general structures shown in Fig. 1 and shows the stepwise yield when they were used to couple nucleotides together and the specific photolysis conditions used..

INS A1

Figure 3 shows the synthesis of 5'-TEMPOC-T-Phosporamidite.

Figure 4 shows the synthesis of NINOC-T-CEP.

Figure 5 shows the synthesis of Me2NPOC-T-CEP. CEP stands for cyanoethyl N, N diisopropyl phosphoramidite.

Figure 6 shows the synthesis of Me3NPOC-T-CEP.

Figure 7 shows the synthesis of NP2NPOC-T-CEP.

Figure 8 shows the synthesis of NA1BOC-T-CEP.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein.

The term "alkyl" refers to a branched or straight chain acyclic, monovalent saturated hydrocarbon radical of one to twenty carbon atoms.

The term "alkenyl" refers to an unsaturated hydrocarbon radical which contains at least one carbon-carbon double bond and includes straight chain, branched chain and cyclic radicals.

The term "alkynyl" refers to an unsaturated hydrocarbon radical which contains at least one carbon-carbon triple bond and includes straight chain, branched chain and cyclic radicals.

The term "aryl" refers to an aromatic monovalent carbocyclic radical having a single ring (e.g., phenyl) or two condensed rings (e.g., naphthyl), which can optionally be

mono-, di-, or tri-substituted, independently, with alkyl, lower-alkyl, cycloalkyl, hydroxylower-alkyl, aminolower-alkyl, hydroxyl, thiol, amino, halo, nitro, lower-alkylthio, lower-alkoxy, mono-lower-alkylamino, di-lower-alkylamino, acyl, hydroxycarbonyl, lower-alkoxycarbonyl, hydroxysulfonyl, lower-alkoxysulfonyl, lower-alkylsulfonyl, lower-alkylsulfinyl, trifluoromethyl, cyano, tetrazoyl, carbamoyl, lower-alkylcarbamoyl, and di-lower-alkylcarbamoyl. Alternatively, two adjacent positions of the aromatic ring may be substituted with a methylenedioxy or ethylenedioxy group. Typically, electron-donating substituents are preferred.

The term "heteroaromatic" refers to an aromatic monovalent mono- or poly-cyclic radical having at least one heteroatom within the ring, *e.g.*, nitrogen, oxygen or sulfur, wherein the aromatic ring can optionally be mono-, di- or tri-substituted, independently, with alkyl, lower- alkyl, cycloalkyl, hydroxylower-alkyl, aminolower-alkyl, hydroxyl, thiol, amino, halo, nitro, lower-alkylthio, lower-alkoxy, mono-lower-alkylamino, di-lower-alkylamino, acyl, hydroxycarbonyl, lower-alkoxycarbonyl, hydroxysulfonyl, lower-alkoxysulfonyl, lower-alkylsulfonyl, lower-alkylsulfinyl, trifluoromethyl, cyano, tetrazoyl, carbamoyl, lower-alkylcarbamoyl, and di-lower-alkylcarbamoyl. For example, typical heteroaryl groups with one or more nitrogen atoms are tetrazoyl, pyridyl (*e.g.*, 4-pyridyl, 3-pyridyl, 2-pyridyl), pyrrolyl (*e.g.*, 2-pyrrolyl, 2-(N-alkyl)pyrrolyl), pyridazinyl, quinolyl (*e.g.* 2-quinolyl, 3-quinolyl etc.), imidazolyl, isoquinolyl, pyrazolyl, pyrazinyl, pyrimidinyl, pyridonyl or pyridazinonyl; typical oxygen heteroaryl radicals with an oxygen atom are 2-furyl, 3-furyl or benzofuranyl; typical sulfur heteroaryl radicals are thienyl, and benzothienyl; typical mixed heteroatom heteroaryl radicals are furazanyl and phenothiazinyl. Further the term also includes instances where a heteroatom within the ring has been oxidized, such as, for example, to form an N-oxide or sulfone.

The term "optionally substituted" refers to the presence or lack thereof of a substituent on the group being defined. When substitution is present the group may be mono-, di- or tri-substituted, independently, with alkyl, lower-alkyl, cycloalkyl, hydroxylower-alkyl, aminolower-alkyl, hydroxyl, thiol, amino, halo, nitro, lower-alkylthio, lower-alkoxy, mono-lower-alkylamino, di-lower-alkylamino, acyl, hydroxycarbonyl, lower-alkoxycarbonyl, hydroxysulfonyl, lower-alkoxysulfonyl, lower-alkylsulfonyl, lower-alkylsulfinyl, trifluoromethyl, cyano, tetrazoyl, carbamoyl, lower-alkylcarbamoyl, and di-lower-alkylcarbamoyl. Typically, electron-donating substituents such as alkyl, lower-alkyl, cycloalkyl, hydroxylower-alkyl, aminolower-alkyl, hydroxyl, thiol, amino, halo,

lower-alkylthio, lower-alkoxy, mono-lower-alkylamino and di-lower-alkylamino are preferred.

The term "electron donating group" refers to a radical group that has a lesser affinity for electrons than a hydrogen atom would if it occupied the same position in the molecule. For example, typical electron donating groups are hydroxy, alkoxy (e.g. methoxy), amino, alkylamino and dialkylamin0.

The term "leaving group" means a group capable of being displaced by a nucleophile in a chemical reaction, for example halo, nitrophenoxy, pentafluorophenoxy, alkyl sulfonates (*e.g.*, methanesulfonate), aryl sulfonates, phosphates, sulfonic acid, sulfonic acid salts, and the like.

"Activating group" refers to those groups which, when attached to a particular functional group or reactive site, render that site more reactive toward covalent bond formation with a second functional group or reactive site. The group of activating groups which are useful for a carboxylic acid include simple ester groups and anhydrides. The ester groups include alkyl, aryl and alkenyl esters and in particular such groups as 4-nitrophenyl, N-hydroxylsuccinimide and pentafluorophenol. Other activating groups are known to those of skill in the art.

"Chemical library" or "array" is an intentionally created collection of differing molecules which can be prepared either synthetically or biosynthetically and screened for biological activity in a variety of different formats (*e.g.*, libraries of soluble molecules; and libraries of compounds tethered to resin beads, silica chips, or other solid supports). The term is also intended to refer to an intentionally created collection of stereoisomers.

"Predefined region" refers to a localized area on a solid support which is, was, or is intended to be used for formation of a selected molecule and is otherwise referred to herein in the alternative as a "selected" region. The predefined region may have any convenient shape, *e.g.*, circular, rectangular, elliptical, wedge-shaped, etc. For the sake of brevity herein, "predefined regions" are sometimes referred to simply as "regions." In some embodiments, a predefined region and, therefore, the area upon which each distinct compound is synthesized smaller than about 1 cm^2 or less than 1 mm^2. Within these regions, the molecule synthesized therein is preferably synthesized in a substantially pure form. In additional embodiments, a predefined region can be achieved by physically separating the regions (*i.e.*, beads, resins, gels, etc.) into wells, trays, etc.

"Solid support", "support", and "substrate" refer to a material or group of materials having a rigid or semi-rigid surface or surfaces. In many embodiments, at least one surface of the solid support will be substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different compounds with, for example, wells, raised regions, pins, etched trenches, or the like. According to other embodiments, the solid support(s) will take the form of beads, resins, gels, microspheres, or other geometric configurations.

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer (preparative) chromatography, distillation, or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by references to the examples hereinbelow. However, other equivalent separation or isolation procedures can, or course, also be used.

A "channel block" is a material having a plurality of grooves or recessed regions on a surface thereof. The grooves or recessed regions may take on a variety of geometric configurations, including but not limited to stripes, circles, serpentine paths, or the like. Channel blocks may be prepared in a variety of manners, including etching silicon blocks, molding or pressing polymers, etc.

This invention provides novel compounds which are useful for providing protecting groups in chemical synthesis, preferably in the solid phase synthesis of oligonucleotides and polypeptides and high density arrays thereof. These compounds are generally photolabile and comprise protecting groups which can be removed by photolysis to unmask a reactive group. Specifically, the preferred compounds are shown in Figures 1 and 9. More specifically, the preferred compounds have R or R1 groups which can be H, optionally substituted alkyl, alkenyl, alknyl, aryl, or heteroaromatic groups.

Preferably, M will be a monomeric building block that can be used to make a macromolecule. Such building blocks include amino acids, nucleic acids, nucleotides, nucleosides, monosaccharides and the like. Preferred nucleosides are deoxyadenosine, deoxycytidine, thymidine and deoxyguanosine as well as oligonucleotides incorporating such nucleosides. Preferably, the building block is linked to the photolabile protecting group via a hydroxy or amine group. When nucleotide and oligonucleotide compositions are used, with the protecting groups of this invention, the protecting groups are preferably

incorporated into the 3'-OH or the 5'-OH of the nucleoside. Other preferred compounds are protected peptides, proteins, oligonucleotides and oligodeoxynucleotides. Small organic molecules, proteins, hormones, antibodies and other such species having nucleophilic reactive groups can be protected using the protecting groups disclosed herein.

The use of nucleoside and nucleotide analogs is also contemplated by this invention to provide oligonucleotide or oligonucleoside analogs bearing the protecting groups disclosed herein. Thus the terms nucleoside, nucleotide, deoxynucleoside and deoxynucleotide generally include analogs such as those described herein. These analogs are those molecules having some structural features in common with a naturally occurring nucleoside or nucleotide such that when incorporated into an oligonucleotide or oligonucleoside sequence, they allow hybridization with a naturally occurring oligonucleotide sequence in solution. Typically, these analogs are derived from naturally occurring nucleosides and nucleotides by replacing and/or modifying the base, the ribose or the phosphodiester moiety. The changes can be tailor made to stabilize or destabilize hybrid formation or enhance the specificity of hybridization with a complementary nucleic acid sequence as desired.

Analogs also include protected and/or modified monomers as are conventionally used in oligonucleotide synthesis. As one of skill in the art is well aware oligonucleotide synthesis uses a variety of base-protected deoxynucleoside derivatives in which one or more of the nitrogens of the purine and pyrimidine moiety are protected by groups such as dimethoxytrityl, benzyl, tert-butyl, isobutyl and the like. Specific monomeric building blocks which are encompassed by this invention include base protected deoxynucleoside H-phosphonates and deoxynucleoside phosphoramidites.

For instance, structural groups are optionally added to the ribose or base of a nucleoside for incorporation into an oligonucleotide, such as a methyl, propyl or allyl group at the 2'-0 position on the ribose, or a fluoro group which substitutes for the 2'-O group, or a bromo group on the ribonucleoside base. 2'-O-methyloligoribonucleotides (2'-O-MeORNs) have a higher afinity for complementary nucleic acids (especially RNA) than their unmodified counterparts. 2'-0-MeORNA phosphoramidite monomers are available commercially, *e.g.*, from Chem Genes Corp. or Glen Research, Inc. Alternatively, deazapurines and deazapyrimidines in which one or more N atoms of the purine or pyrimidine heterocyclic ring are replaced by C atoms can also be used.

The phosphodiester linkage, or "sugar-phosphate backbone" of the oligonucleotide analogue can also be substituted or modified, for instance with methyl phosphonates or O-methyl phosphates. Another example of an oligonucleotide analogue for purposes of this disclosure includes "peptide nucleic acids" in which a polyamide backbone is attached to oligonucleotide bases, or modified oligonucleotide bases. Peptide nucleic acids which comprise a polyamide backbone and the bases found in naturally occurring nucleosides are commercially available.

Nucleotides with modified bases can also be used in this invention. Some examples of base modifications include 2-aminoadenine, 5-methylcytosine, 5-(propyn-1-yl)cytosine, 5-(propyn-1-yl)uracil, 5-bromouracil, and 5-bromocytosine which can be incorporated into oligonucleotides in order to increase binding affinity for complementary nucleic acids. Groups can also be linked to various positions on the nucleoside sugar ring or on the purine or pyrimidine rings which may stabilize the duplex by electrostatic interactions with the negatively charged phosphate backbone, or through hydrogen bonding interactions in the major and minor groves. For example, adenosine and guanosine nucleotides can be substituted at the N^2 position with an imidazolyl propyl group, increasing duplex stability. Universal base analogues such as 3-nitropyrrole and 5-nitroindole can also be included. A variety of modified oligonucleotides and oligonucleotide analogs suitable for use in this invention are described "Antisense Research and Applications", S.T. Crooke and B. LeBleu (eds.) (CRC Press, 1993) and "Carbohydrate Modifications in Antisense Research" in ACS Symp. Ser. #580, Y.S. Sanghvi and P.D. Cook (eds.) ACS, Washington, D.C. 1994).

Compounds of this invention can be prepared by carbonylating an alcohol or amine precursor “Y” with a carbonylation reagent such as for example, phosgene ($COCl_2$), carbonyldiimidazole or pentafluorophenoxy chloroformate and the like to provide Y-C(O)-X where X is a leaving group derived from the carbonylating reagent (Cl, if phosgene was used, pentafluorophenoxy, if pentafluorophenoxy chloroformate was used, etc.). This intermediate, Y-C(O)-X is then reacted with a molecule M carrying a nucleophilic group whose protection is desired to yield a protected building block Y-C(O)-M.

Alternatively, one may first carbonylate the group on the molecule being protected with a carbonylation reagent, such as one described above, and subsequently displace the leaving group X thus inserted with the hydroxyl group of the aromatic carbinol. In either procedure, one frequently uses a base such as triethylamine or diisopropylethylamine and the like to facilitate the displacement of the leaving group.

One of skill in the art will recognize that the protecting groups disclosed herein can also be attached to species not traditionally considered as "molecules". Therefore, compositions such as solid surfaces (*e.g.*, paper, nitrocellulose, glass, polystyrene, silicon, modified silicon, GaAs, silica and the like), gels (*e.g.*, agarose, sepharose, polyacrylamide and the like to which the protecting groups disclosed herein are attached are also contemplated by this invention.

The protecting groups of this invention are typically removed by photolysis, i.e. by irradiation, though in selected cases it may be advantageous to use acid or base catalyzed cleavage conditions. The synthesis can occur in either the 3'>5' or 5'>3' directions. Generally irradiation is at wavelengths greater than about 350 nm, preferably at about 365 nm. The photolysis is usually conducted in the presence of hydroxylic solvents, such as aqueous, alcoholic or mixed aqueous-alcoholic or mixed aqueous-organic solvent mixtures. Alcoholic solvents frequently used include methanol and ethanol. The photolysis medium may also include nucleophilic scavengers such as hydrogen peroxide. Photolysis is frequently conducted at neutral or basic pH.

This invention also provides a method of attaching a molecule with a reactive site to a support, comprising the steps of:

(a) providing a support with a reactive site;

(b) binding a molecule to the reactive site, said first molecule comprising a masked reactive site attached to a photolabile protecting group of the formula Y-C(O)-, and

(c) removing the photolabile protecting group to provide a derivatized support comprising the molecule with an unmasked reactive site immobilized thereon.

As one of skill will recognize, the process can be repeated to generate a compound comprising a chain of component molecules attached to the solid support. In a "mix and match" approach, the photolabile protecting groups may be varied at different steps in the process depending on the ease of synthesis of the protected precursor molecule. Alternatively, photolabile protecting groups can be used in some steps of the synthesis and chemically labile (e.g. acid or base sensitive groups) can be used in other steps, depending for example on the availability of the component monomers, the sensitivity of the substrate and the like. This method can also be generalized to be used in preparing arrays of compounds, each compound being attached to a different and identifiable site on the support as is disclosed in U.S. Patent Nos. 5,143,854, 5,384,261, 5,424,186 5,445,934, 6,022963 and

copending U.S. Patent Application, Serial No. 08/376,963, filed January 23, 1995, incorporated for reference for all purposes in their entireties.

Thus, a related aspect of this invention provides a method of forming, from component molecules, a plurality of compounds on a support, each compound occupying a separate region of the support, said method comprising the steps of:

(a) activating a region of the support;

(b) binding a molecule to the region, said molecule comprising a masked reactive site linked to a photolabile protecting group of the formula Y-C(O)-, and

(c) repeating steps (a) and (b) on other regions of the support whereby each of said other regions has bound thereto another molecule comprising a masked reactive site linked to the photolabile protecting group, wherein said another molecule may be the same or different from that used in step (b);

(d) removing the photolabile protecting group from one of the molecules bound to one of the regions of the support to provide a region bearing a molecule with an unmasked reactive site;

(e) binding an additional molecule to the molecule with an unmasked reactive site;

(f) repeating steps (d) and (e) on regions of the support until a desired plurality of compounds is formed from the component molecules, each compound occupying separate regions of the support.

A related method of forming a plurality of compounds on predefined regions of a support involves binding a molecule with a reactive site protected with a chemically labile protecting group to an activated region of the support and chemically removing the chemically labile protecting group to reveal the reactive site. The reactive site is then protected with a photolabile protecting group of this invention. This process is repeated for other regions of the support with other molecules as desired to provide a support having molecules with reactive sites protected by photolabile protecting groups on separate regions of the support. Reactive sites can be unmasked by removing the photolabile group from selected regions and coupled to additional molecules with photolabile protecting groups as described earlier to build up arrays of compounds on the support. Again, in a "mix and match" approach, monomers with chemically labile protecting groups can be attached to a reactive site on the substrate (i.e., on the support itself when the first layer of monomers is being assembled or subsequently onto an already attached monomer whose reactive site has

been unmasked) and these chemically labile protecting groups can be replaced by a photolabile protecting groups of this invention. The replacement is accomplished by removing the chemically labile protecting group under conditions that do not affect any photolabile groups which may be on the support. This then reveals an unmasked reactive site on the monomer which had carried the chemically labile protecting group and this unmasked reactive site is reacted with a reagent of the formula Y-C(O)-X, where X is a leaving group. Thereby, this region of the support is protected by a photolabile protecting group which can be selectively removed by light directed systems described in U.S. Patent Nos. 5,143,854, 5,384,261, 5,424,186 and 5,445,934 and further described below (incorporated by reference in their entireties for all purposes). This method is particularly useful when the monomers are more readily available carrying chemically labile protecting groups than the photolabile protecting groups described herein. It will be recognized that any method of forming a chain of compounds or an array of compounds on a support using in at least one step a protecting group/reagent or compound of this invention is within the scope of the methods this invention.

Generally, these methods involve sequential addition of monomers to build up an array of polymeric species on a support by activating predefined regions of a substrate or solid support and then contacting the substrate with a protected monomer of this invention (*e.g.*, a protected nucleoside or amino acid). It will be recognized that the individual monomers can be varied from step to step. A common support is a glass or silica substrate as is used in semiconductor devices.

The predefined regions can be activated with a light source, typically shown through a screen such as a photolithographic mask similar to the techniques used in integrated circuit fabrication. Other regions of the support remain inactive because they are blocked by the mask from illumination and remain chemically protected. Thus, a light pattern defines which regions of the support react with a given monomer. The protected monomer reacts with the activated regions and is immobilized therein. The protecting group is removed by photolysis and washed off with unreacted monomer. By repeatedly activating different sets of predefined regions and contacting different monomer solutions with the substrate, a diverse array of polymers of known composition at defined regions of the substrate can be prepared. Arrays of 10^{6}, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12} or more different polymers can be assembled on the substrate. The regions may be 1 mm^2 or larger, typically 10 μm^2 and may be as small as 1 μm^2.

The methods described herein may also employ component molecules comprising a masked reactive site attached to a photolabile protecting group having the structure Y. In such cases, the protecting group is attached to an acidic reactive site, such as a carboxylate or phophate and is removed by photolysis.

The solid substrate or solid support may be of any form, although they preferably will be planar and transparent (and potentially some three dimensional structure). The supports need not necessarily be homogenous in size, shape or composition, although the supports usually and preferably will be uniform. In some embodiments, supports that are very uniform in size may be particularly preferred. In another embodiment, two or more distinctly different populations of solid supports may be used for certain purposes.

Solid supports may consist of many materials, limited primarily by capacity for derivatization to attach any of a number of chemically reactive groups and compatibility with the synthetic chemistry used to produce the array and, in some embodiments, the methods used for tag attachment and/or synthesis. Suitable support materials typically will be the type of material commonly used in peptide and polymer synthesis and include glass, latex, heavily cross-linked polystyrene or similar polymers, gold or other colloidal metal particles, and other materials known to those skilled in the art. The chemically reactive groups with which such solid supports may be derivatized are those commonly used for solid phase synthesis of the polymer and thus will be well known to those skilled in the art, *i.e.*, carboxyls, amines, and hydroxyls.

To improve washing efficiencies, one can employ nonporous supports or other solid supports less porous than typical peptide synthesis supports; however, for certain applications of the invention, quite porous beads, resins, or other supports work well and are often preferable. One such support is a resin in the form of beads. In general, the bead size is in the range of 1 nm to 100 μm, but a more massive solid support of up to 1 mm in size may sometimes be used. Particularly preferred resins include Sasrin resin (a polystyrene resin available from Bachem Bioscience, Switzerland); and TentaGel S AC, TentaGel PHB, or TentaGel S NH_2 resin (polystyrene-polyethylene glycol copolymer resins available from Rappe Polymere, Tubingen, Germany). Other preferred supports are commercially available and described by Novabiochem, La Jolla, California.

In other embodiments, the solid substrate is flat, or alternatively, may take on alternative surface configurations. For example, the solid substrate may contain raised or depressed regions on which synthesis takes place. In some embodiments, the solid substrate

will be chosen to provide appropriate light-absorbing characteristics. For example, the substrate may be a polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO_2, SiN_4, modified silicon, or any one of a variety of gels or polymers such as (poly)tetrafluorethylene, (poly)vinylidendifluoride, polystyrene, polycarbonate, or combinations thereof. Other suitable solid substrate material will be readily apparent to those of skill in the art. Preferably, the surface of the solid substrate will contain reactive groups, which could be carboxyl, amino, hydroxyl, thiol, or the like. More preferably, the surface will be optically transparent and will have surface Si-OH functionalities, such as are found on silica surfaces.

The photolabile protecting groups and protected monomers disclosed herein can also be used in bead based methods of immobilization of arrays of molecules on solid supports.

A general approach for bead based synthesis is described in copending application Serial Nos. 07/762,522 (filed September 18, 1991); 07/946,239 (filed September 16, 1992); 08/146,886 (filed November 2, 1993); 07/876,792 (filed April 29, 1992) and PCT/US93/04145 (filed April 28, 1993), Lam et al. (1991) *Nature* **354**:82-84; PCT application no. 92/00091 and Houghten et al, (1991) *Nature* **354**:84-86, each of which is incorporated herein by reference for all purposes.

Other methods of immobilization of arrays of molecules in which the photocleavable protecting groups of this invention can be used include pin based arrays and flow channel and spotting methods.

Photocleavable arrays also can be prepared using the pin approach developed by Geysen et al. for combinatorial solid-phase peptide synthesis. A description of this method is offered by Geysen et al., *J. Immunol. Meth.* (1987) **102:**259-274, incorporated herein by reference.

Additional methods applicable to library synthesis on a single substrate are described in U.S. Patent Nos. 5,384,261, 5,677,195, 6,040,193 that are hereby incorporated by reference in their entireties for all purposes. In the methods disclosed in these applications, reagents are delivered to the substrate by either (1) flowing within a channel defined on predefined regions or (2) "spotting" on predefined regions. However, other approaches, as well as combinations of spotting and flowing, may be employed. In each instance, certain activated regions of the substrate are mechanically separated from other regions when the monomer solutions are delivered to the various reaction sites.

Photocleavable linkers are particularly suitable for this technology as this delivery method may otherwise result in poor synthesis fidelity due to spreading, reagent dilution, inaccurate delivery, and the like. By using a photocleavable linker, rather than a conventional acid-cleavable linker, the purest material can be selectively cleaved from the surface for subsequent assaying or other procedures. More specifically, masks can be used when cleaving the linker to ensure that only linker in the center of the delivery area (*i.e.*, the area where reagent delivery is most consistent and reproducible) is cleaved. Accordingly, the material thus selectively cleaved will be of higher purity than if the material were taken from the entire surface.

Typically, the molecules used in this method will be the monomeric components of complex macromolecules. These monomeric components can be small ligand molecules, amino acids, nucleic acids, nucleotides, nucleosides, monosaccharides and the like, thereby allowing one to synthesize arrays of complex macromolecules or polymeric sequences, such as polypeptides, nucleic acids and synthetic receptors, on the solid support.

EXAMPLE

Examples of the preferred groups shown in Figure 2 were synthesized and tested as 5'-photolabile protecting groups on thymidine phosporamidite monomers. Surface photolysis rates in different solvents (std. 365nm lightsource) were determined as described elsewhere (McGall et al., JACS 1997, 119: 5081, hereby incorporated by reference in its entirety for all purposes). Standard coupling efficiency measurements were made using the cleavable linker HPLC analysis technique (see U.S.S.No. 09/545,207, and attorney docket no. 3233.1, which are both hereby incorporated by reference in their entireties).

Figure 1 shows the preferred compounds and their synthesis. It shows the general structures of the preferred structures, the preferred structures, their synthesis, the yields of the nucleic acid sequences formed using the preferred protecting groups, and the photolysis conditions. Also, the synthesis steps are annotated with references that relate to the specific synthesis. All of these references are hereby incorporated by reference in their entireties for all purposes.

5'-TEMPOC-T-Phosphoramidite was synthesized using the steps outlined in Fig. 3 and the details shown in the references in that Figure. Specifically, the following references are hereby incorporated by reference in their entireties for all purposes as well as the steps that are cited: Dyer, et al. JOC 64:7988 (1999); Tetrahedron Lett., 38(52), 8933-4 (1997);

Mcgall, et al., JACS 119:5081 (1997). The Fig. indicates that triphosgene may work equally well for step #1 and that chloroformate could probably be used without purification in step #2. NINOC-T-CEP was synthesized according to the steps shown in Fig. 4 and the following references are incorporated by reference in their entireties for all purposes as well as the steps that are cited; Bromidge, et al. (1998) J. Med. Chem. 41: 1598; Brooker, LS, et al. (1953) U.S. Patent No. 2,646,430; Boekelheide, et al. (1954) J. Org. Chem. 19: 504; Bennet, et al. (1941) J. Chem. Soc. 74:244; and Mortensen, et al. (1996) Org. Prep. Proc. Int. 28: 123. Figs. 5-8 show the synthesis of the following compounds; Me2NPOC-T-CEP; Me3NPOC-T-CEP; and NA1BOC-T-CEP. Fig. 8 refers to Aust. J. Chem 48:1969-70 which is also incorporated by reference in its entirety. Abbreviations used in the first step of the processes indicate the source of the material. For example, DAV is Davos, LAN is Lancaster, ALH is Adrich. CEP stands for cyanoethyl N, N diisopropyl phosphoramidite.

The foregoing invention has been described in some detail by way of illustration and examples, for purposes of clarity and understanding. It will be obvious to one of skill in the art that changes and modifications may be practiced within the scope of the appended claims. Therefore, it is to be understood that the above description is intended to be illustrative and not restrictive. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the following appended claims, along with the full scope of equivalents to which such claims are entitled.

All patents, patent applications and publications cited in this application are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual patent, patent application or publication were so individually denoted.